

Listing of Claims

1. (Currently Amended) A method of diagnosing decreased vascular function in a subject that does not have symptomatic cardiovascular disease, comprising
selecting a subject that does not have symptomatic cardiovascular disease;
assaying the number of endothelial progenitor cells in a blood sample from the subject;
comparing the number of endothelial progenitor cells in the blood sample from the subject with a control, wherein the control is a number of endothelial progenitor cells in a blood sample from a control subject that does not have symptomatic cardiovascular disease and has a low Framingham Risk Score, and

identifying a subject with increased cardiovascular risk or decreased vascular function, wherein a decrease in the number of endothelial progenitor cells in the sample as compared to the control indicates decreased vascular function in the subject, and wherein vascular function is vascular contractility, brachial reactivity or a combination thereof.

2. (Previously Presented) The method of claim 1, wherein assaying the number of endothelial progenitor cells comprises
isolating the buffy coat from a blood sample of the subject;
isolating peripheral blood mononuclear cells from the buffy coat;
culturing the peripheral blood mononuclear cells on a solid support coated with a first substrate, wherein cells from the peripheral blood mononuclear cells adhere to the first substrate;
isolating non-adherent cells;
culturing the non-adherent cells on a solid support coated with a second substrate, wherein a subset of the non-adherent cells adhere to the second substrate and form colonies;
confirming that the subset of the non-adherent cells that adhered to the second substrate are endothelial progenitor cells by immunological assessment; and
counting the number of colonies on the solid support,
wherein the first substrate, or the second substrate, or both the first substrate and the second substrate comprise fibronectin, vitronectin, or collagen.

3. (Currently Amended) The method of claim 2, wherein a lower number of colonies on the solid support as compared to a control indicates decreased vascular function wherein vascular function is vascular contractility, brachial reactivity or a combination thereof.

4. (Original) The method of claim 1, wherein assaying the number of endothelial progenitor cells comprises
determining the number of VEGFR²⁺CD31^{hi} cells in the sample.

5. (Original) The method of claim 1, wherein the control is a blood sample from a subject that does not have atherosclerosis.

6. (Original) The method of claim 1, wherein the control is a standard value.

7. (Original) The method of claim 2, wherein the first substrate comprises fibronectin.

8. (Original) The method of claim 2, wherein the first and the second substrate comprise fibronectin.

9. (Currently Amended) A method of diagnosing increased vascular function in a subject, comprising

selecting a subject that does not have symptomatic cardiovascular disease;

assaying a number of endothelial progenitor cells in first and second blood samples taken from the subject, wherein the second blood sample is taken from the subject after the first blood sample is taken from the subject;

comparing the number of endothelial progenitor cells in the first blood sample with the number of endothelial progenitor cells in the second blood sample, and

identifying a subject with increased vascular function, wherein an increase in the number of endothelial progenitor cells in the second blood sample as compared to the first blood sample indicates increased vascular function in the subject, wherein vascular function is vascular contractility, brachial reactivity or a combination thereof.

10. (Original) The method of claim 9, wherein the subject has been treated with a cholesterol-lowering agent.

11. (Previously Presented) The method of claim 10, wherein the first blood sample is a blood sample taken from the subject prior to treatment with the cholesterol-lowering agent.

12. (Previously Presented) The method of claim 9, wherein assaying the number of endothelial progenitor cells comprises

isolating the buffy coat from a blood sample of the subject;

isolating peripheral blood mononuclear cells from the buffy coat;

culturing the peripheral blood mononuclear cells on a solid support coated with a first substrate, wherein cells from the peripheral blood mononuclear cells adhere to the first substrate;

isolating non-adherent cells that do not adhere to the first substrate;

culturing the non-adherent cells on a solid support coated with a second substrate, wherein a subset of the non-adherent cells adhere to the second substrate and form colonies;

confirming that the subset of the non-adherent cells that adhered to the second substrate are endothelial progenitor cells; and

counting the number of colonies on the solid support,

wherein the first substrate, or the second substrate, or both the first substrate and the second substrate comprise fibronectin, vitronectin, or collagen.

13. (Currently Amended) The method of claim 12, wherein a higher number of colonies on the solid support as compared to a control indicates increased vascular function, wherein vascular function is vascular contractility, brachial reactivity or a combination thereof.

14. (Original) The method of claim 12, wherein the first substrate comprises fibronectin.

15. (Original) The method of claim 12, wherein the first substrate and the second substrate comprises fibronectin.

16. (Original) The method of claim 9, wherein assaying the number of endothelial progenitor cells comprises

determining the number of VEGFR²⁺CD31^{hi} cells in the sample.

17-19. (Canceled).

20-47. (Canceled).

48. (Previously Presented) A method of diagnosing increased cardiovascular risk or decreased vascular function in a subject, comprising

selecting a subject that does not have symptomatic cardiovascular disease;

assaying a number of senescent endothelial progenitor cells in a blood sample from the subject, wherein a senescent endothelial progenitor cell is a viable endothelial cell that exhibits clonal exhaustion in vitro;

comparing the number of senescent endothelial progenitor cells in the blood sample from the subject with a control, wherein the control is a number of senescent endothelial progenitor cells in a blood sample from a control subject that does not have

symptomatic cardiovascular disease and has a low Framingham Risk Score, and

identifying a subject with increased cardiovascular risk or decreased vascular function, wherein an increase in the number of senescent endothelial progenitor cells in the sample as compared to the control indicates increased cardiovascular risk or decreased vascular function.

49. (Original) The method of claim 48, wherein the control is a standard value.

50. (Original) The method of claim 48, wherein the control is a number of senescent endothelial progenitor cells in a blood sample from a subject known not to be affected by a disease or disorder.

51-53. (Canceled).

54. (Previously Presented) The method of claim 1, wherein vascular function comprises

vascular contractility, brachial reactivity, atrial hyperplasia, or a combination thereof.

55. (Previously Presented) The method of claim 1, wherein the control subject has a Framingham Risk Score of less than 1.5.

56. (Previously Presented) The method of claim 2, wherein confirming that the subset of the non-adherent cells that adhere to the second substrate are endothelial progenitor cells by immunological assessment comprises contacting the cells with antibodies that specifically bind Vascular Endothelial Growth Factor Receptor kinase insert domain receptor, contacting the cells with antibodies that specifically bind CD31, or measuring uptake of DiI-acetylated low density lipoprotein and co-staining with BS-1 Lectin.

57. (Previously Presented) The method of claim 12, wherein confirming that the subset of the non-adherent cells that adhere to the second substrate are endothelial progenitor cells comprises immunostaining with antibodies that specifically bind Vascular Endothelial Growth Factor Receptor kinase insert domain receptor; contacting the cells with antibodies that specifically bind CD31; or measuring uptake of DiI-acetylated low density lipoprotein followed by co-staining with BS-1 Lectin.

58. (Previously Presented) The method of claim 48, wherein the control subject has a Framingham Risk Score of less than 1.5.

59. (Previously Presented) The method of claim 48, assaying a number of senescent endothelial progenitor cells comprises measuring endogenous beta-galactosidase.